# CARBON DIOXIDE SEQUESTRATION BY ALGAE IN POME MEDIUM

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# ABSTRACT

Carbon dioxide ($CO_2$) has been identified as one of the primary greenhouse gases (GHG) in the atmosphere. Carbon dioxide emissions have increased by about 35% since 1990 and cause the global warming. In Malaysia, this problem is due to the rapid growth of palm oil industries. A lot of carbon dioxide is emitted in producing crude palm kernel oil. Besides, palm oil mill effluent (POME) is the waste discharged by the palm oil industries with high biochemical oxygen demand (BOD), which has created serious water pollution in Malaysia, but it contains micronutrient need for microalgae growth. $CO_2$ emission can be reduced through $CO_2$ sequestration by photoautotrophic algae through photosynthesis. In this study, microalgae (*Chlorella* sp.) was cultured in POME medium in conical flasks and the interaction effect of light intensity and $CO_2$ concentration in the sparged mixture of air and $CO_2$ on algae growth (expressed as cell dry weight and specific growth rate) were identified. Then the results obtained from this experiment were analyzed with 2-level factorial design by using Design Expert 6.0.8 Software. In this experiment, the light intensity was supplied by fluorescent lamps at 2,000-12,000 lux. Meanwhile, the microalgae culture was sparged by 5 L/min of air mixture with $CO_2$ concentration (2-10% v/v). From this study, it was found that the increasing of light intensity and the increasing of $CO_2$ concentration gives the highest cell dry weight. At low light intensity, cell dry weight decreased even the $CO_2$ increased. The interaction between light intensity and $CO_2$ concentration in cell dry weight production was insignificant. In response to specific growth rate of *Chlorella* sp., at low $CO_2$ concentration, increasing light intensity did not affect the specific growth rate. On the other hand, when $CO_2$ concentration was high, increasing light intensity would reduce the specific growth rate of *Chlorella* sp. Both factors (light intensity and $CO_2$ concentration) were identified as the significant in effecting specific growth rate of *Chlorella* sp. As conclusion, since the $CO_2$ sequestration was measured based on the cell dry weight produce, light intensity was the significant factor for $CO_2$ sequestration by *Chlorella* sp. in POME medium. Nevertheless, too high a light intensity would cause photo-inhibition and reduced the growth rate of Chlorella sp. which in turn slows down the process of $CO_2$ sequestration.

# ABSTRAK

Karbon dioksida telah dikenal pasti sebagai salah satu daripada gas-gas rumah hijau utama di atmosfera. Pengeluaran karbon dioksida telah meningkat sebanyak kira-kira 35% sejak tahun 1990 dan menyebabkan pemanasan global. Di Malaysia, masalah ini adalah disebabkan oleh pertumbuhan pesat industri minyak sawit. Banyak karbon dioksida dilepaskan dalam menghasilkan minyak mentah isirong sawit. Selain itu, sisa kumbahan kilang minyak sawit adalah sisa yang dilepaskan oleh industri minyak sawit dengan permintaan oksigen biokimia yang tinggi, yang telah mewujudkan pencemaran air yang serius di Malaysia, tetapi ia mengandungi mikronutrien yang diperlukan untuk pertumbuhan mikroalgae. Pelepasan gas karbon dioksida boleh dikurangkan melalui pemencilan karbon dioksida oleh photoautotrophic algae melalui fotosintesis. Dalam kajian ini, mikroalgae (*Chlorella* sp.) telah dikultur dengan sisa kumbahan kilang minyak kelapa sawit di dalam kelalang kon dan kesan interaksi keamatan cahaya dan kepekatan karbon dioksoda di dalam campuran udara dan gas karbon dioksida kepada pertumbuhan algae (dinyatakan sebagai berat sel kering dan kadar pertumbuhan spesifik) telah dikenal pasti. Kemudian keputusan yang diperolehi dari eksperimen ini telah dianalisis dengan 2-level factorial design menggunakan perisian Design Expert 6.0.8. Dalam eksperimen ini, keamatan cahaya dibekalkan oleh lampu neon di 2,000-12,000 lux. Sementara itu, kultur mikroalgae itu dibekalkan sebanyak 5 L/min campuran udara dengan kepekatan karbon dioksida (2-10% v/v). Daripada kajian ini, didapati bahawa peningkatan keamatan cahaya dan peningkatan kepekatan karbon dioksida memberikan berat sel kering yang tertinggi. Pada keamatan cahaya yang rendah, berat sel kering menurun walaupun karbon dioksida meningkat. Interaksi antara keamatan cahaya dan kepekatan karbon dioksida dalam penghasilan berat sel kering tidak penting. Sebagai tindak balas kepada kadar pertumbuhan spesifik *Chlorella* sp., Pada kepekatan karbon dioksida yang rendah, peningkatan keamatan cahaya tidak menjejaskan kadar pertumbuhan spesifik. Sebaliknya, apabila kepekatan karbon dioksida adalah tinggi, peningkatan keamatan cahaya akan mengurangkan kadar pertumbuhan spesifik *Chlorella* sp. Kedua-dua faktor ( keamatan cahaya dan kepekatan karbon dioksida) telah dikenal pasti sebagai faktor yang penting pada kadar pertumbuhan spesifik *Chlorella* sp. Kesimpulannya, kerana pemencilan karbon dioksida telah diukur berdasarkan berat sel kering, keamatan cahaya merupakan faktor penting bagi pemencilan karbon dioksida oleh *Chlorella* sp. dalam medium POME. Walau-bagaimanapun, keamatan cahaya yang terlalu tinggi akan menyebabkan perencatan fotosintesis dan mengurangkan kadar pertumbuhan *Chlorella* sp. yang seterusnya melambatkan proses pemencilan karbon dioksida.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| μ | Specific growth rate |
| BBM | Bold's Basal Medium |
| BOD | Biochemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| DOE | Design of Experiment |
| GHG | Greenhouse Gas |
| lux | SI unit of illumination intensity |
| pH | Hydrogen Ion Concentration |
| POME | Palm Oil Mill Effluent |
| rpm | rotation per minute |
| RSM | Response Surface Method |
| RuBisCO | Ribulose-1,5-biphosphate carboxylase oxygenase |
| sp. | Species |

# 1 INTRODUCTION

## *1.1 Motivation and statement of problem*

Carbon dioxide ($CO_2$) has been identified as one of the primary greenhouse gases (GHG) in the atmosphere (Choo and Subramaniam, 2012). Carbon dioxide emissions have increased by about 35% since 1990 (Kaladharan *et al*., 2009). Koorosh and Adal (2012) stated that the increasing of carbon dioxide emissions causes the global warming which leads to acid rain and air pollution. Lam and Lee (2011) said that in Malaysia this problem is due to the rapid growth of palm oil industries. Malaysia has become the second largest palm oil producer in the world and its palm oil industry served as a backbone to Malaysia economy. However, in order to produce crude palm kernel oil a lot of carbon dioxide is emitted (Choo and Subramaniam, 2012). In addition, it also contributes to others negative impacts to environment such as deforestation and habitat destruction when a new oil palm plantation is started or a new palm oil mill is built.

$CO_2$ emission can be reduced through $CO_2$ sequestration, which is the process of carbon capture from atmosphere (Kenneth, 2010). A recent study found that photoautotrophic algae have the potential to sequestrate $CO_2$ through carbon fixation during photosynthesis (Kaladharan *et al*., 2009). Peter *et al*. (2006) summarized the advantages of using microalgae as its rapid growth, its ability to produce more biofuel than oil plants, its ability to sequester $CO_2$, the low toxicity of biodegradable fuel without sulfur, it is not being involved in competition with food crops, and also it is not being involved in natural habitat destruction. In addition, microalgae are rich in micronutrients. Table 1.1 lists the micronutrients that containing in microalgae.

Table 1.1: Micronutrient contain in microalgae (Peter *et al*., 2006).

| **Micronutrient** | **Content (%)** |
|---|---|
| **Protein** | 25-40 |
| **Carbohydrate** | 5-30 |
| **Lipid/oils** | 10-30 |

Microalgae need certain condition to grow. They needs light, nutrients and a warm condition. It can be grown in wastewater treatment ponds, animal waste and other liquid waste. Nitrogen, phosphorus, potassium and magnesium are the nutrients needed for microalgae growth. According to Choong (2012), palm oil mill effluent (POME) contains significant amounts of these nutrients and can be used as the medium for microalgae culture.

POME is the waste discharged by the palm oil industries which has created serious water pollution in Malaysia (Habib *et al.*, 1997). According to Choong (2012) POME consists of palm fruit water soluble components and suspended materials such as palm fibre and oil residue. Since POME is acidic and contains residual oil that difficult to separate, it cannot be discharged without first being treated. It needs a lot of oxygen to decompose completely. This phenomenon is known as high biochemical oxygen demand (BOD). Sometimes, BOD of POME is up to 100 times higher than domestic sewage. Table 1.2 shows the characteristics of POME and discharged limit.

Table 1.2: Characteristics of POME and discharged limit (Official Portal Malaysian Palm Oil Board, December 2012)

| **Parameter** | **POME (range)** | **POME (mean)** | **Discharge standard (1.1.1984 and thereafter)** |
|---|---|---|---|
| **Temperature ($^{o}$C)** | 80-90 | 85 | 45 |
| **pH** | 3.4-5.2 | 42 | 5.0-9.0 |
| **Oil and grease** | 130-18,000 | 6,000 | 50 |
| **BOD** | 10, 250-43,750 | 25,000 | 100 |
| **COD** | 15,000-100,000 | 51,000 | - |
| **Total solid** | 11,500-79,000 | 40,000 | - |
| **Suspended solid** | 5,000-54,000 | 18,000 | 400 |
| **Total volatile solid** | 9,000-72,000 | 34,000 | - |
| **Total nitrogen** | 180-1,400 | 750 | 200 |
| **Ammoniacal nitrogen** | 4-80 | 35 | 150 |

During photosynthesis, microalgae will absorb $CO_2$ and release oxygen. So that, microalgae will sequestrate the $CO_2$ emission during the production of crude palm kernel oil. Meanwhile, oxygen that is released by microalgae during photosynthesis will be used for POME decomposition. This correlation will reduce the pollution caused by palm oil industries.

Therefore, the aim of this research is to sequestrate the $CO_2$ that release during the production of crude palm kernel oil by culturing microalgae in POME medium which have high BOD and chemical oxygen demand (COD). The usage of microalgae will provide oxygen for POME decomposition.

## *1.2 Objectives*

The following are the objectives of this research:

- To study the factors of light intensity and % (v/v) $CO_2$ sequestration that are influencing the $CO_2$ sequestration of algae in POME medium at shake flask scale.

## *1.3 Scope of this research*

The following are the scope of this research:

i) To study the effect of light intensity (2,000-12,000 lux) and % (v/v) $CO_2$ (2-10%) on the growth of *Chlorella* sp. in POME medium.

ii) To study the interaction between these two factors using Design Expert 6.0.8 software.

## *1.4 Main contribution of this work*

By doing this research, after considering the parameter of light intensity and $CO_2$ concentration, the interaction factors affecting the $CO_2$ sequestration can be achieved by using Design Expert 6.0.8 Software. Besides, this process also can provide the oxygen for POME decomposition. The cost of $CO_2$ sequestration can be reduced since it does not need any other costly method for $CO_2$ sequestration like $CO_2$ injection into soil which is practically used in Canada. Other $CO_2$ sequestration methods need high cost to set up the technology and transportation. The $CO_2$ sequestration by microalgae is biologically method, which also known as win-win strategies. The microalgae can get nutrients source that containing in POME which will cause water pollution if release directly to environment without treatment.

# 2 LITERATURE REVIEW

## *2.1 Carbon Dioxide ($CO_2$) Sequestration*

This As a means of reducing the emission of carbon dioxide ($CO_2$) and other greenhouse gases (GHGs) such as methane ($CH_4$) and nitrous oxide ($N_2O$) in atmosphere, various carbon sequestration methods have been investigated. Many sequestration strategies have focused on expensive carbon capture technologies. Oelkers and Cole (2008) proposed three major types of carbon storage: geological storage, ocean storage, and mineral carbonation. Geological storage is the process of injection of $CO_2$ into porous rock formations (Holloway, 2001). Meanwhile, ocean storage means the injection of captured $CO_2$ into the ocean, which usually greater than 1000 meters in depths to make it isolated from the atmosphere (Adams and Caldeira, 2008). Mineral carbonation was aims to create stable carbonate minerals, such as magnesite ($MgCO_3$) and calcite ($CaCO_3$),by reacting $CO_2$ with silicate minerals containing magnesium and calcium (Oelkers *et al*. 2008). However, these processes need high cost to set up the technology and transportation to sequestration site.

In Canada, $CO_2$ injection into soil reservoir has been applied. This method has improved the economics because of the value added component associated with incremental hydrocarbon recovery. But, the problem is in the selecting of the right underground reservoir for storing $CO_2$ and it has the maximum level of $CO_2$ can be stored in the reservoir (Koorosh and Adal, 2012).

Recent study by Kaladharan *et al*. (2009) found that microalgae have the potential to sequestrate the $CO_2$ from atmosphere. Microalgae are the primary producers and it plays an important role in carbon sequestration. Some microalgae species are grown to optimize $CO_2$ sequestration. $CO_2$ is used as the carbon source in an autotrophic culture (Kenneth, 2010). Microalgae will sequester $CO_2$ from the atmosphere to form biomass (Kenneth, 2010). Since the molecular formula of microalgae biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, approximately half of the dry weight of algal biomass is carbon

(Chisti, 2007). Based on mass balance, the sequestration value can be quantified; for every pound of algal biomass created, 1.83 pounds of CO2 are sequestered (Chisti, 2007). Biomass is produced according to the following reversible reaction (Chaumont, 1993):

$$6.14\,CO_2 + 3.65\,H_2O + NH_3 \xleftrightarrow{\text{Light}} C_{6.14}H_{10.3}O_{2.24}N + 6.85\,O_2 \quad (2.1)$$

## *2.2 Microalgae*

Microalgae are single cell organisms which represent both bacteria and eukaryotes (Lam & Lee, 2011). Microalgae species are divided into four categories depending on their pigmentation, life cycle and basic cellular structure: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), blue-green algae (*Cyanophyceae*) and golden algae (*Chrysophyceae*) (Khan *et al.*, 2009). William and Laurens (2010) have identified the microalgae main components which are carbohydrates, proteins, nucleic acids and lipids (typically phospholipids and glycolipids).

The advantages of using microalgae as $CO_2$ sequester: (1) rapid growth rate; (2) as a source of biofuels; (3) low toxicity of biodegradable fuel without sulfur; (4) not being involved in natural habitat destruction; (5) rich in micronutrients; (6) synthesize and accumulate large quantities of neutral lipids/oil; (7) oil yield exceed the yield of the best oilseed crops; (8) can be cultivated in saline/brackish water/coastal seawater or non-arable land; (9) does not being involved in competition with food crops; (10) utilize nitrogen and phosphorus from a variety of wastewater sources (11) produce value-added by-products (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed and fertilizer); (12) do not need herbicide and pesticide; and (13) higher biomass productivity (Peter *et al*., 2006; Khan *et al*., 2009; Tredici, 2010).

Microalgae are said to be rapid growth rate because microalgae can double their biomass in less than 24 hours (Tredici, 2010). Moreover, once in every 3-4 hr

microalgae are able to divide its cell under favorable growing conditions (Williams and Laurens, 2010). Khan *et al*. (2009) said that microalgae have ability to uptake the large amount of nutrients from water sources because of their simple cellular structure and large surface to volume ratio. This promotes their growth rate. In addition, when grown in open pond culture system, the algae can be grown in wastewater treatment systems using the waste water stream effluent as a water and nutrient source.

*Chlorella* sp. (Figure 2.1) was used in this study. There are several advantages of using *Chlorella* sp. This is because *Chlorella* sp. can produce high biomass under high $CO_2$ concentration due to its ability to fix up to 74% of the original $CO_2$ with only 2 seconds of $CO_2$ residence time (Sebastian *et al*., 2013). Sebastian *et al*. (2013) also stated that under ambient air concentration of $CO_2$ (0.037%) *Chlorella* sp. will growth rapidly. Besides, *Chlorella* sp. can reduce the BOD value. Violeta *et al*. (2011), in their studies, found that BOD was reduced more than 87.1% during the growth of *Chlorella* sp. For application, *Chlorella* sp. usually used in cultivation of marine organism for human food, and for zooplanktons culture in marine fish hatchery (Igor, 2001).

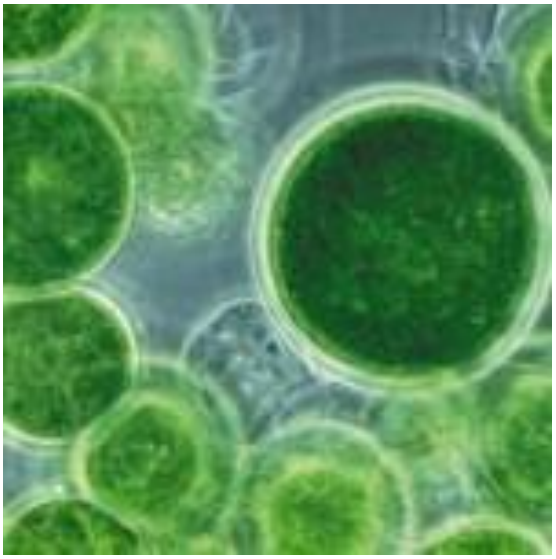

Figure 2.1: *Chlorella* sp.

## *2.3 Microalgae Growth Condition*

### 2.3.1 Carbon dioxide

Carbon dioxide ($CO_2$) is the carbon source for the process of photosynthesis by microalgae (Ryu*et al*., 2012). During photosynthesis, $CO_2$ is dissolved in water and becomes $HCO_3^-$, which was absorbed by microalgae (Ciferrum, 1983). Hence, $CO_2$ are significant key factors for microalgae cultivation and balances the $CO_2$ ecosystem as well. However, each microalga shows different behaviour towards $CO_2$ consumption. Some microalgae required higher $CO_2$ concentration to avoid binding of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to Oxygen rather than $CO_2$ (Rahaman *et al*., 2011), meanwhile some microalgae growth restricted by increasing injection rate of $CO_2$ into the culture medium.

With regards to this research, in order to maximizing $CO_2$ uptake by microalgae, $CO_2$ were supplied at different volumetric flowrate so that the effect of $CO_2$ towards microalgae growth can be observed throughout the study.The recommended range for $CO_2$ concentration for *Chlorella* sp. culture is 2-10% (Mariana *et al*., 2013).

### 2.3.2 pH

pH range for microalgae cultivation is 7–9, with the optimum range being 8.2–8.7 (Coutteau, 1996). The pH value must be maintained because too low or too high pH value will cause disruptions of microalgae cells and leads to the death of culture, the increase in pH occurs over the time in very dense cultures condition (Sabrettin,2012).

### 2.3.3 Temperature

Most common cultured temperature of microalgae is between 20 to 30˚C (Chisti, 2007). But, it is depending on the culture medium composition, the species and strain cultured

(Woertz*et al*., 2004). Exceeding the optimum temperature 2-4˚C will result in the total loss of culture (Mata *et al*., 2010).

### 2.3.4 Aeration

Mixing is necessary to provide turbulent flow (Chisti, 2007). This turbulent flow is provide to prevent sedimentation of the algae, to ensure that all cells are equally exposed to light and nutrients, to avoid thermal stratification and facilitate gas transfer rate between the culture medium and the air (Mata *et al*., 2010). Besides, aeration is functioning to strip out accumulated oxygen because when the level of oxygen dissolved greater than air saturation value, it will inhibit the photosynthesis (Chisti, 2007).

### 2.3.5 Nutrient

Growth medium must contain the inorganic elements that constitute the algal cell. The essential elements are nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) (Peter *et al*., 2006). Furthermore, Chisti (2007) also recommended adding the iron (Fe) and silicon (Si) as the nutrient for microalgae growth.

### 2.3.6 Light

Algae are affected by the light intensities to which they are exposed. Exposure to too little light will decrease biomass productivity because it has prohibited logarithmic growth. Exposure to too much light inhibits growth and kills the organism. This is known as photo-inhibition (Esra *et al*., 2007). Photo-inhibition means that there is no further growth in an algal culture as a result of increasing light intensities. Photo-inhibition results when maximum growth rate is achieved given an unchanging suite of nutrient conditions. Up to the light saturation value, algae will grow exponentially with increasing irradiance. Above this light saturation value, a further increase in irradiance actually reduces the biomass growth rate. Most algae become photo-inhibited at irradiance levels slightly greater than the light level at which their growth rate peaks

(Karcher, 2010). Previous study by Igor (2001) found that the suitable range of light level for *Chlorella* sp. culture is 2,000-12,000 lux.

## *2.4 Palm Oil Mill Effluent (POME)*

POME is a thick brownish colloidal suspension containing mixture of water (95–96%), oil (0.6–0.7%) and total solids (4–5%), including 2–4% of suspended solids (Wu *et al*., 2007). The suspended solid is originating from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater in a ratio of 9:15:1, respectively (Wu *et al*., 2010). Since no chemical are added during extraction process, it is classified as non-toxic effluent (Khalid and Wan Mustafa, 1992). POME is discharged with high temperature (80-90$^{o}$C), acidic (pH around 4.5), high biochemical oxygen demand (BOD), high chemical oxygen demand (COD), contain oil, grease and suspended solids (Khalid and Wan Mustafa, 1992; Md Din *et al*., 2006; Wu *et al*., 2007). Therefore, the palm oil mill industry in Malaysia has been identified as one of the industries that have contributed to the water pollution throughout the country. As stated by Lam and Lee (2011), based on 17.56 million tons of the total crude palm oil production in year 2009, it was estimated about 8 million $m^3$ (11,600 million gallon) of POME was generated from Malaysian palm oil mills. Table 2.1 shows the typical characteristics of POME. From Table 2.1, the total nitrogen contents are still high compared to the discharge standard for wastewater, which is 200 mg/L (Lam & Lee, 2011). If the POME is discharged untreated, it can certainly cause considerable environmental problems. Meanwhile, nitrogen source is one of the nutrients needed to promote microalgae growth. The basic nitrate concentration required to grow microalgae effectively is in the range of 200–400 mg/L (Li *et al*., 2008). Other minerals that are required for microalgae growth, such as Fe, Zn, P, Mg, Ca and K, are also present in POME (Habib *et al*., 1998).Thus, POME provide an alternative option as a chemical remediation to grow microalgae for biomass production.

Table 2.1: Characteristics of sterilization condensate, separator sludge and hydrocyclone wastewater (Whiting, 1978).

| Parameters[a] | Sterilizer condensate | Separator sludge | Hydrocyclone wastewater |
|---|---|---|---|
| **pH** | 5.0 | 4.5 | - |
| **BOD[b]** | 23000 | 29000 | 5000 |
| **COD** | 47000 | 64000 | 15000 |
| **TSS** | 5000 | 23000 | 7000 |
| **Total dissolved solid** | 34000 | 22000 | 100 |
| **Total nitrogen** | 600 | 1200 | 100 |
| **Ammoniacal nitrogen** | 20 | 40 | - |
| **Oil and grease** | 4000 | 7000 | 300 |

a All parameters are in units of mg/l except pH.

b The sample for BOD analysis is incubated at 30 $^{o}$C for 3 days.

## *2.5 Bold's Basal Medium (BBM)*

Several media such as CFTRI media, OFERR media, Revised media, Bangladesh medium No (3), Zarrouk's media and Bold's Basal Media (BBM) has been identified as microalgae culture medium. According to Sankar and Ramasubramanian (2012), *Chlorella* sp. grown best in Bold's Basal Media compared to others. Microalgae growth is expressed as concentration of microalgae biomass. From Table 2.2, we can conclude that Bold's Basal Medium give higher biomass production. This is due to nutrients contains in the medium. In this study, Bold's Basal Medium was used to culture the stock microalgae and preparing inoculums. Table 2.2 below shows the experimental result conducted by Sankar and Ramasubramanian (2012).

Table 2.2: Chlorophyll b content in (mg/L) *C.vulgaris* cultured in six different types of culture media (Sankar and Ramasubramanian, 2012)

| **Culture Days (Days)** | **BBM (mg/L)** | **Bangladesh M3 (mg/L)** | **Revised M6 (mg/L)** | **OFERR M (mg/L)** | **CFTRI M (mg/L)** | **Zarrouk's M (mg/L)** |
|---|---|---|---|---|---|---|
| **5** | 0.35 | 0.33 | 0.23 | 0.20 | 0.11 | 0.06 |
| **10** | 0.47 | 0.37 | 0.31 | 0.26 | 0.21 | 0.18 |
| **15** | 0.50 | 0.47 | 0.38 | 0.34 | 0.26 | 0.20 |
| **20** | 0.56 | 0.51 | 0.40 | 0.37 | 0.32 | 0.30 |

Each value is expressed as mean ± SEM (n=9) X Statistically significant at $P<0.05$.

## *2.6 Mathematical Analysis*

### 2.6.1 Factorial Design

The experimental variables, factors and interaction effects on the responses can be investigated by using a factorial design (Zatilfarihiah *et al*., 2009). 2-level factorial design ($2^n$) is commonly used in mathematical analysis. The maximum result based on statistical principles with the less number of experiments can be identified from this method (Mullai *et al*., 2010). From this method, the number of experiment need to be conducted can be calculated. The factorial experiments make use the Yates' Method (Yates, 1937) to analyze the main effects and the interactive effects. Main effect can be categorized as independent variables where all the effects based on the experimental error. Meanwhile, failure of one factor to show the same result on the response at different level of other factor was actually interaction. Thus, the interactive effect was for two or more variables that dependent between them (Ding, 2011).

### 2.6.1 Yates' Method

This mathematical method was used in the factorial experiment. Yates' method is used to analyze on the main effects and interactive effects. Based on Yates' method calculation, it will show the yield response surface whether it is curved or uncurved, whether flat, increasing or decreasing based on one or more of experimental variables and the direction. In order to detect the effect of difference on the yields, the experimental variables were under two levels equidistant from the centre point (Ding, 2011). Yet, advance analysis using Design of Experiment (DOE) software such as Design Expert Software was developed in order to simplify the analysis.

DOE software was developed with the purpose of providing many powerful statistical tools such as two-level factorial screening designs, general factorial studies, response surface methods (RSM), mixture design techniques and combinations of process factors, mixture components, and categorical factors.

# 3 MATERIALS AND METHODS

## *3.1 Flowchart of Research Methodology*

The brief description of process flow for $CO_2$ sequestration by algae in POME medium is shown in Figure 3.1.

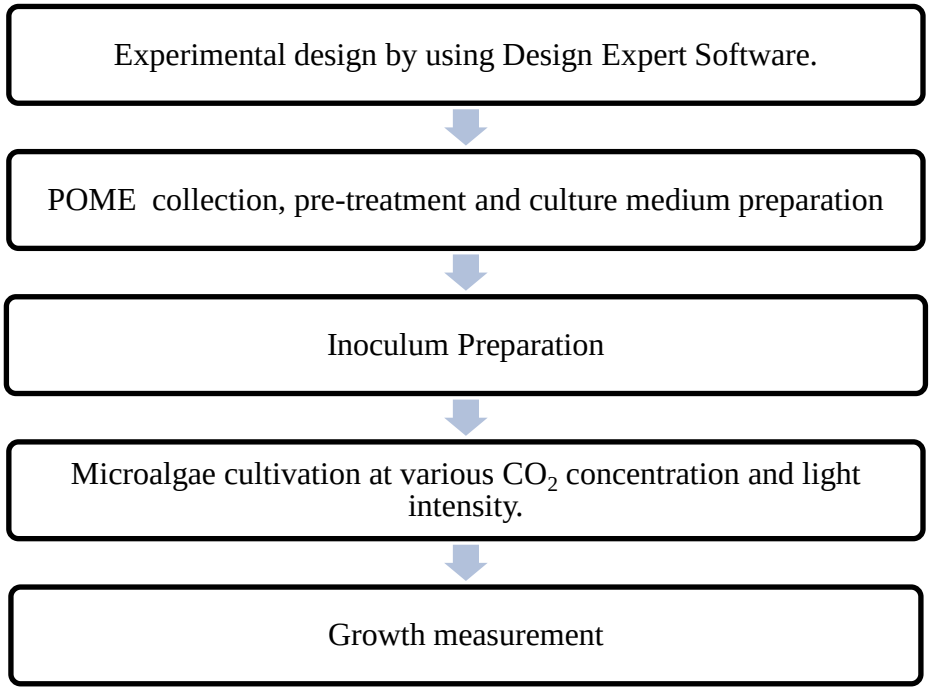


Figure 3.1: Process flowchart on $CO_2$ sequestration process.

## *3.2 Experimental Design*

Factorial experiment was design by using Design Expert Software. The parameter range identified was 2,000-12,000 lux for light intensity and 2-10% for $CO_2$ concentration. A $2^2$ full factorial design with 3 centre points was chosen. The experimental runs were generated by Design Expert version 6.0.8 and shown in Table 3.1

Table 3.1: Factorial Experimental Design

| No of Experimental Run | Light Intensity (lux) | $CO_2$ Concentration (%) |
|---|---|---|
| **1** | 12,000 | 10 |
| **2** | 7,000 | 6 |
| **3** | 2,000 | 10 |
| **4** | 2,000 | 2 |
| **5** | 12,000 | 2 |
| **6** | 7,000 | 6 |
| **7** | 7,000 | 6 |

## *3.3 POME Collection and pre-treatment*

Culture medium of POME from polishing pond was obtained from Dominion Square Palm Oil Mill Sdn. Bhd.,Gambang, Pahang, Malaysia. POME was treated first by removing the solid particle by centrifugation (Eppendorf Refrigerated Centrifuge model 5810R) at 10,000 rpm for 10 minutes. Then, POME was autoclaved (Hiraclave, Hirayama, model PH PM088) at 121$^{o}$C for 20 minutes to prevent contamination.

## *3.4 Bold's Basal Medium (BBM)*

### 3.4.1 Preparation of stock solution for Bold's Basal Medium (BBM)

Medium was prepared in aqueous solution. Three stock solutions were prepared separately by using ultrapure water to prevent contamination. All chemicals used are

indicated in Table 3.2. To fully dissolve all chemicals, preparation should be done at 50-60$^{o}$C under stirring condition.

Table 3.2: List of chemicals used to prepare BBM stock solution (Daphnia Research group, 2007).

| **Stock Solution** | **Chemical Name** | **Formula** | **Weight (g)** | **Ultrapure Water (mL)** |
|---|---|---|---|---|
| A | Dipotassium Phosphate | $K_2HPO_4$ | 1.875 | 250 |
| | Potassium dihydrogen phosphate | $KH_2PO_4$ | 4.375 | |
| | Magnesium sulphate heptahydrate | $MgSO_4.7H_2O$ | 1.875 | |
| | Sodium Nitrate | $NaNO_3$ | 6.250 | |
| | Calcium chloride dehydrate | $CaCl_2.2H_2O$ | 0.625 | |
| | Sodium Chloride | NaCl | 0.625 | |
| B | Ethylenediaminetetraacetic acid tetrasodium salt | EDTA - $Na_4$ | 5.000 | 100 |
| | Potassium hydroxide | KOH | 3.100 | |
| | Ferrous sulphate heptahydrate | $FeSO_4.7H_2O$ | 0.498 | |
| | Sulphuric acid conc. (wt per mL = 1.84g) | $H_2SO_4$ | 0.1mL | |
| | Boric acid | $H_3BO_3$ | 1.142 | |
| C | Zinc sulphate heptahydrate | $ZnSO_4.7H_2O$ | 0.353 | 25 |
| | Manganese chloride tetrahydrate | $MnCl_2.4H_2O$ | 0.058 | |
| | Copper (II) sulphate pentahydrate | $CuSO_4.5H_2O$ | 0.063 | |
| | Cobalt (II) nitrate hexahydrate | $Co(NO_3)_2.6H_2O$ | 0.020 | |
| | Sodium molybdate (VI) dehydrate | $Na_2MoO_4.2H_2O$ | 0.048 | |